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Effect of Fylloton application on photosynthetic activity of Moldavian dragonhead (*Dracocephalum moldavica* L.)

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Abstract

The field experiment was carried out in 2014 in Perespa, Poland to examine the impact of biostimulant Fylloton on the photosynthetic activity of Moldavian dragonhead cv 'Alba'. Moldavian dragonhead seeds were sown in April at a depth of 1-1.5 cm, with the spacing 45 x 20 cm. During the growing season, Fylloton was foliar-applied at a dose 2 l/ha (0.7%) and 3 l/ha (1%) by single spraying or double spraying of plants. One week after application of biostimulator, the photosynthetic activity of plants was measured. Use of Fylloton had a positive impact on the efficiency of photosynthetic apparatus and chlorophyll content in the leaves of Moldavian dragonhead plants. Fylloton is environmentally friendly preparation and helps to improve the efficiency of photosynthetic apparatus. It should therefore be recommended for use in sustainable agriculture.

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Keywords: amino acids; *Dracocephalum moldavica*; *Lamiaceae*; photosynthesis; seaweed; biostimulant.

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1. Introduction

Plant cultivation technologies friendly to humans and the environment are intensively searched for in sustainable agriculture. The increasing demand for food and plant products determines the development of crop production and the actions aimed at obtaining high yields of good quality crops. One of the elements which allow to obtain the expected production effects are biostimulants or formulations based on organic or inorganic compounds naturally occurring in the environment, eventually synthetic. Their mechanism of action is based on the induction of plant resistance mechanisms to stress factors like pathogens, pests and adverse weather conditions. Therefore these substances contribute to improve the amount and quality of the crop plants. Natural biostimulators usually contain free amino acids, extracts from seaweed or fruit, effective microorganisms, humic substances or chitosan. Many studies prove that biostimulants positively affect various metabolism processes controlling plant growth, development and productivity (Calvo et al., 2014; Kocira et al., 2013; Kocira et al., 2015; Przybysz et al., 2014).

In biostimulants, components activating metabolic processes in plants may be present alone or in conjunction with micro, macro- or other active ingredients. It seems that the combination of active ingredients is more effective in stimulating the metabolic processes in the plant, and therefore in the presented studies our attention is focused on biostimulator Fylloton. It is based on the compounds naturally occurring in the environment such as extract of seaweed (*Ascophyllum nodosum*) and free amino acids of vegetable origin obtained in the enzymatic hydrolysis. Until now research on the combined use of seaweed extract and amino acids are rare (Norouzpour and Abad, 2013; Pulk, 2015).

Moreover, no reports exist on use of such extracts on medicinal plants. Therefore, it seems appropriate to examine the impact of biostimulator Fylloton on the photosynthetic activity of Moldavian dragonhead cv 'Alba' (*Dracocephalum moldavica* L.).

2. Materials and Methods

2.1. Plant materials and growth conditions

The study was carried out in 2014 in Perespa (50°66′N; 23°63′E), Poland. The soil type was characterized as Brown Rendzina belonging to the Rendzinas soil group. It is alkaline (pH in 1M KCl around 7.4–7.5) and rich in phosphorus, potassium, and magnesium. The experiment was established in a randomized block design in four replications with an elementary experimental plot area of 5 m^2. Moldavian dragonhead seeds were sown in the third 10-day period of April at a depth of 1-1.5 cm, with the spacing 45 x 20 cm. During the growing season, Fylloton was foliar-applied at a dose 2 l/ha (0.7%) and 3 l/ha (1%) by single spraying (at the 10-12 leaf stage – 20th June) and double spraying of plants (at the 10-12 leaf stage - 20th June and at the beginning of plant blooming – 4th July). This was applied with a GARLAND FUM 12B battery field sprayer (Lecher LU 120–03) at a pressure of 0.30 MPa, using 300 l liquid per hectare. The following experimental variants were carried out: control: where plants were treated with the same volume of water (no biostimulator was applied), 1- or 2-fold spraying with 0.7% Fylloton and 1- or 2 fold spraying with 1% Fylloton. All the results were compared to control. Tillage for plants was done using good agricultural practices. No pesticides were used (pests did not exceed the thresholds of harmfulness).

2.2. Photosynthetic activity of plants

During the growing season, one week after application of biostimulator, the photosynthetic activity of plants was measured. In the case of a single spraying the second measurement was carried out in the second term at the appropriate plant development stage. To estimate the actual photochemical activity of PSII *in situ*, chlorophyll *a* fluorescence in plants was measured using a pulse amplitude-modulated (PAM) fluorimeter (Mini PAM, Walz, GmbH, FRG, Germany) with a light emitting diode at 650 nm and a standard intensity 0.15 μmol $m^{-2}s^{-1}$ PAR (photosynthetically active radiation). A Leaf-Clip holder 2030-B was used during all the measurements. Standard settings optimized for measurements with leaf samples at approx. 12 mm distance between fiberoptics and leaf surface were used for measurements. The parameters listed hereinafter were set. Measuring light: on; Measuring pulse frequency: 0.6 KHz; Actinic illumination time: 30s; Actinic light intensity: 5 (relative intensity 5.5); Actinic light factor: 1.00; LC-WIDTH: 10s; LC INT: 3; Int-Temp: on; Light gain: 1.00; Temp. gain: 1.00; Saturating light pulse width: 0.8; Saturation pulse intensity: 8; Electronic signal damping: 2 (0.2s); Measuring light intensity: 8. The Auto-Zero function was applied in order to suppress any unavoidable background signal. All the measurements were done under field condition with the assumption that a photon flux density is below saturation level. The following fluorescence parameters were measured: F - the present fluorescence yield, M - fluorescence after saturation pulse was applied, $Yield = (M - F)/M$, PAR-photosynthetically active radiation, ETR-electron transfer rate calculated as: $ETR = Yield * PAR * ETR - factor$; *i.e.* $ETR = Yield * PAR * 0.5 * 0.84$. The standard factor 0.84 corresponds to the fraction of incident light absorbed by a leaf.

Chlorophylls and a nitrogen status N were estimated by Chlorophyll Meter SPAD-502 Plus (Konica Minolta) (ten plants of each experimental combination).

2.3. Statistical analysis

Data on photosynthetic parameters of four replicates of each combination were subjected to statistical analysis. The Shapiro-Wilk test was performed for the normal distribution of data. The results were analyzed using one-way analysis of variance, ANOVA. The significance of differences between evaluated mean values was estimated by means of Turkey's test intervals of confidence at a significance level of $p < 0.05$. Statistical analysis was performed with Statistica 12 (StatSoft, Inc.).

3. Results and Discussions

Fluorescence measurements carried out in the first term (I measurement) showed that the single use of 0.7 % solution of Fylloton resulted in the 3 - fold increase of photosynthetically active radiation (PAR) used by plants, as compared with 2-fold spraying with biostimulant of a higher concentration (Table 1). This parameter is however difficult to interpret as it corresponds to the amount of energy captured by plant at the exact time of measurement. Although no visible difference in weather condition, the differences can occur on molecular level. One should also consider the condition of the plant itself (energy levels in the photosynthetic apparatus). On the other hand, the increase of this parameter can indirectly inform about the utilization of the solar energy by plant. This parameter is accompanied by a parameter F which corresponds to the maximum fluorescence yield of a sample under natural/field conditions. The increase of F entails the decrease in PAR. Generally, this parameter informs about a fraction of an energy, which in the moment of experiment is not used during photosynthesis. Fm' is a maximum fluorescence yield of illuminated sample. Based on this basic information, various fluorescence parameters have been defined that have proven useful for characterization of photosynthetic performance. Particularly useful expressions were derived for the maximal Photosystem II (PS II) quantum yield of dark-adapted samples, the effective PS II quantum yield of illuminated samples, $Y(II) = (Fm'-F)/Fm' = \Delta F/Fm'$ (Genty et al., 1989). Y(II) corresponds to the fraction of energy that is photochemically converted in PS II (used in photosynthesis).

Table 1. Effect of Fylloton on the PS II chlorophyll fluorescence and photosynthetically active radiation of Moldavian dragonhead (*Dracocephalum moldavica* L.) plants.

Experimental variant	F		Fm'		PAR	
	I	II	I	II	I	II
Control	399.63a	364.14bc	1028.50a	1434.43ab	449.25ab	78.57ab
Single spraying with 0.7% Fylloton	376.63a	286.29a	1018.75a	1686.00bc	875.75b	57.00a
Double spraying with 0.7% Fylloton	501.88a	315.43ab	1266.00a	1411.29a	304.63ab	124.86b
Single spraying with 1% Fylloton	371.88a	308.00ab	1039.00a	1764.71c	629.88ab	48.43a
Double spraying with 1% Fylloton	339.50a	384.57c	1061.00a	1752.71c	270.00a	117.43b

I – measurement at the 10-12 leaf stage; II - measurement at the beginning of plant blossoming.

Means in the same column with different letters are significantly different (α=0.05).

An increase of 11 and 12% of the effective photochemical yield of PS II (Y (II)) was obtained after a single application of Fylloton as compared to the control, independent of the concentration (for the 1% and 0.7 % solution of biostimulant) (Table 2). Although no statistical differences, an increase in this parameter was observed for all the experimental variants as compared to control.

The highest electron transport rate (ETR) was obtained during I-st measurement in a single - sprayed plants with a lower concentration of Fylloton. The three-fold increase of this parameter was observed as compared to the double use of the biostimulant formulation at a lower concentration. It is worth of notice that the highest ETR rates were registered for plants at the 10-12 leaf stage which means the period of intensive growth as compared to the period of blooming. Interestingly, double application of Fylloton results in the increase of ETR parameter during blooming as compared to control.

Foliar application of Fylloton resulted in the increase of the content of chlorophyll in the leaves of Moldavian dragonhead (for first and second measurement) as compared to control. A similar result was reported after application of Atonik biostimulator on cotton, *Arabidopsis thaliana,* oilseed rape, and common osier (Djanaguiraman et al., 2009; Przybysz et al., 2014; Wróbel and Woźniak, 2008). Both increase in the yield Y(II) and SPAD parameter indicates no need for applying N-fertilizer.

Leaf chlorophyll content is often well correlated with leaf photosynthetic rates (Percival et al. 2008). Correlations between SPAD values and F-Fm'/Fm' values as measures of photosystem II efficiency are limited. The F-Fm'/Fm' ratio is positively correlated to the PSII quantum yield and an indirect measurement of plant physiologic status for which values of 0.8 ± 0.05 correspond to highly efficient use of the excitation energy in photochemical processes. In our study such a value was observed for the experimental variant where a single spraying with Fylloton was applied (II term). F-Fm'/Fm' ratios of 0.6-0.8 were associated with SPAD values between 49 and 62 in this study.

Numerous authors reported that biostimulant-treated plants increase the intensity of photosynthesis on basil, cotton, rocket, *Arabidopsis thaliana,* oilseed rape and common osier (Borowski and Blamowski, 2009; Djanaguiraman et al., 2009; Janas 2011; Przybysz et al., 2014; Wróbel and Woźniak, 2008).

Table 2. Effect of Fylloton on the photosynthetic and chlorophyll parameters and of Moldavian dragonhead (*Dracocephalum moldavica* L.) plants.

Experimental variant	Y(II)		ETR		SPAD	
	I	II	I	II	I	II
Control	0.597a	0.742a	110.79ab	24.63ab	52.2a	49.8a
Single spraying with 0.7% Fylloton	0.623a	0.830b	222.76b	19.84a	62.6b	54.0ab
Double spraying with 0.7% Fylloton	0.583a	0.771ab	65,10a	39.51b	61.6b	55.9b
Single spraying with 1% Fylloton	0.621a	0.824b	146.69ab	16.64a	59.3b	56.3b
Double spraying with 1% Fylloton	0.665a	0.778ab	73.46a	37.81b	59.2b	57.2b

I – measurement at the 10-12 leaf stage; II - measurement at the beginning of plant blossoming.

Means in the same column with different letters are significantly different (α=0.05).

4. Conclusions

Fylloton affected the photosynthetic activity of Moldavian dragonhead, but the results were dependent on the concentration, frequency of application of biostimulant and on the date of measurement. Photosynthesis is however a very complex process, therefore the improvement of the vitality of plant should be interpreted in terms of increase of both parameters: photosynthetic yield Y (II) and the greenness of plants expressed in SPAD parameter.

Use of Fylloton had a positive impact on the efficiency of photosynthetic apparatus of Moldavian dragonhead plants. A single spraying of plants increased the effective photochemical yield of PS II (Y(II)) (in the second measurement). Use of Fylloton biostimulant affected positively the chlorophyll content in the leaves of Moldavian dragonhead. Fylloton as a product based on sea algae extract and amino acids is environmentally friendly and helps to improve the efficiency of photosynthetic apparatus and should therefore be recommended for use in sustainable agriculture.

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